

displacement amplification or other methods for nucleic acid amplification involving nucleic acid synthesis from a primer. The nucleic acid products containing the sequence-delimiting rNMPs can then be cleaved by treatment with a chemical base or a ribonuclease, and analyzed by methods known in the art to obtain the nucleotide-specific pattern of bands or, provided that a Partial Ribo-substitution Reaction is carried out for each of the four nucleotides, the complete sequence of the nucleic acid. Also, provided that primers with distinguishable non-radioactive labels are used, multiple Partial Ribo-substitution sequencing reactions may be carried out simultaneously in the same reaction mixture and run and read in the same lane of a polyacrylamide gel or capillary tube or other matrix for separating the fragments based on size, as is the case for all of the methods of the present invention described herein.

### C. Discussion

Our results reveal that mutations of tyrosine 639 in T7 RNAP reduce the ability of the polymerase to discriminate between rNTPs and dNTPs. A conservative mutation which removes the tyrosine hydroxyl but retains the phenolic ring (Y639F) exhibits w.t. activity but an average reduction of ~20-fold in the selectivity for dNTPs over rNTPs (Table I). Non-conservative mutations of this tyrosine (Y639A/S) also display decreased rNTP/dNTP discrimination (Table III), but are less active than the w.t. enzyme. Replacement of an rNTP by a dNTP typically reduces Y639F transcript elongation rates by only a factor of two. Tyrosine 639 is conserved in a large number of DNA-directed RNA and DNA polymerases (Delarue, et al., 1990). In DNAP I, mutations of the Y766 to serine and phenylalanine have been characterized

(Polesky, et al., 1989; Carrol, et al., 1991). The Y766S mutation was alone amongst a number of active site mutations characterized in decreasing DNAP I fidelity (increasing miscoding). The Y766F mutation displayed w.t. fidelity and activity. Similarly, the T7 RNAP Y639F mutant displays w.t. kinetics (Bonner, et al., 1992, 1994; Woody, et al., 1994), and the only effect we can identify for this mutation in T7 RNAP is the reduced substrate discrimination reported here. Thus, while T7 RNAP Y639F showed decreased dNTP/rNTP selectivity, it did not exhibit increased miscoding as assessed by incorporation of non-complementary NTPs on homopolymeric templates.

The Y639 T7 RNAP mutations present us with, in one sense, the functional unification of polymerases to go along with their structural unification. The active site of w.t. T7 RNAP is forgiving with regards to template structure (RNA or DNA) or mode of initiation (primed or *de novo*). A mutation which relaxes the substrate selectivity of this polymerase further expands the range of activities which it can display *in vitro*. Depending on the substrates and templates presented to it, the Y639F T7 RNAP can act as an RNA- or DNA-directed RNA or DNA polymerase in primed or *de novo* initiated reactions. Thus it can display a variety of activities normally associated with distinct polymerases, including some entirely novel activities such as *de novo* initiated reverse transcription or mixed dNMP/rNMP polymer synthesis.

#### Example 2: A mutant SP6 RNA Polymerase as a DNA Polymerase

After the observations made above with T7 RNAP, we decided to examine bacteriophage SP6 RNA polymerase to determine whether the DNA synthesis properties observed for the mutant T7 RNAP could, as expected, be extended to other

mutant polymerases. Bacteriophage SP6 is a lytic phage which infects the bacterial species *Salmonella tryphimurium* (Butler and Chamberlin, 1982). SP6 phage resembles *E. coli* phage T7 and their genomes are comparable in size, gene organization and pattern of gene expression (Kassavetis, et al., 1982).

The phage encoded RNA polymerases are very similar in size (Butler and Chamberlin, 1982) and amino acid sequence (Katani, et al., 1987).

The homologous tyrosine at position 639 in T7 RNA polymerase is readily identified at position 631 in SP6 RNA polymerase (Fig. 7). Substitution of tyrosine 631 with phenylalanine in the SP6 RNA polymerase was expected to confer the same phenotypic changes in catalytic properties in this enzyme as were demonstrated for Y639FT7 RNA polymerase (Example 1).

Localized Mutagenesis. Refer to Fig. 7 for a summary of the amino acid and nucleotide sequence surrounding TYR631 in the SP6 RNA polymerase gene. Mutagenesis of the Y631 residue may be accomplished by the method of Kunkel, et al. (1991). Alternatively, one of the many other methods for mutagenesis known to those of skill in the art may be used. The amino acid and nucleotide sequences of the resulting TYR631PHE mutant SP6 RNA polymerase are also given in Fig. 7. As shown, the A residue at position 2 in codon 631 of the SP6 RNA polymerase gene was changed to a T. This results in the loss of the single *NdeI* restriction enzyme site which is present in the wild-type gene, permitting identification of mutant clones.

#### Preparation and Purification of Mutant RNA Polymerase

A single clone was selected in which the *NdeI* site was missing and which expressed SP6 RNA polymerase activity.